THE PERSISTENCE OF THE BACTERIUM SERRATIA MARCESCENS IN THE INTESTINAL TRACTS OF TURTLES

An abstract of a Thesis by
Sister Shirley Russo
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Drake University
Advisor: Dr. Rodney A. Rogers

The problem. The persistence of Serratia marcescens in the intestinal tracts of turtles was investigated.

Procedure. Food pellets were injected with Serratia marcescens and fed to the experimental turtles. The retention time of S. marcescens in the intestinal tracts of three genera of turtles was measured by the recovery of the bacteria in fecal and tank water samples collected every 24 hours and plated on tryptic soy agar and an asparagine enriched agar.

Findings. Serratia marcescens was recovered from 28.3% of the experimental turtles in the first fecal or water samples collected following the introduction of the bacterium into the host turtles. In the fifth fecal or water samples, 1.8% of the turtles showed positive cultures and S. marcescens was not isolated after the fifth sampling time.

Conclusion. Serratia marcescens cannot persist in the intestinal tracts of turtles for a period longer than five days with the average retention time being two days.

Recommendations. Additional study should be undertaken to determine the environmental conditions prevailing in the alimentary tracts of turtles. Attention should be directed toward the isolation, identification and enumeration of the genera of bacteria successfully colonating the digestive tract and the identification of the bactericidal substances operative in the turtle's alimentary tract.

THE PERSISTENCE OF THE BACTERIUM *SERRATIA MARCESCENS* IN THE INTESTINAL TRACTS OF TURTLES

A Thesis

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The School of Graduate Studies

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by

Sister Shirley Russo

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Sister Shirley Russo

Approved by Committee:

Rodney A. Rogers
Chairman

James L. Christiansen

Earl R. Busch

Earle L. Canfield
Dean of the School of Graduate Studies

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INTRODUCTION

The common American Snapping Turtle, Chelydra serpentina serpentina, the western painted turtle, Chrysemys picta belli, and the pet store red-eared turtle, Pseudemys scripta elegans are all well-known reptiles. However bacterial studies of these organisms are surprisingly limited.

Turtles have been used to some extent as biological models to study skin allograft and xenograft rejection (Borysenko, 1969); the phylogeny of the immune response including studies of some physical, chemical and serological characteristics of antibody production (Grey, 1963); hemoglobin analysis (Horton et al., 1972); isolation of arboviruses (Hoff and Trainer, 1973); adrenalectomy and its effect on ion regulation and tissue glycogen (Butler and Knox, 1970); and pituitary regulation of appetite and growth (Brown et al., 1974). These studies are selected illustrations of some of the areas of current research. Bacteriological studies of turtles are lacking with the exception of a study of enteric bacteria in red-eared turtles (McCoy and Seidler, 1973). The major bacteriological emphasis has been on the incidence of Salmonella serotypes.

The reported incidence of salmonellosis in the United States increased three-fold between 1947 and 1963 (Sanders et al., 1965). This significant increase resulted in a greater concern in determining the nonhuman vectors of

salmonellosis. In 1946, the turtle was first reported as a carrier of Salmonella in the United States (McNeil and Hinshaw, 1946). In 1952, Boycott, Taylor and Douglas, working in England, reaffirmed the potential relationship of turtles to human salmonellosis. Another decade passed before the first turtle-associated case of salmonellosis was recorded in England (Hersey and Mason, 1963). In 1966, Kaufmann and Morrison conducted an epidemiologic study of turtle-associated salmonellosis and reported over fifty cases in the United States. This disclosure launched widespread investigations in state health departments throughout the nation resulting in the adoption of federal and state legislation requiring Salmonella-free certification or banning the sale of Pseudemys scripta elegans in U.S. pet stores.

In 1973 the bacterial study of red-eared turtles was extended to the isolation, enumeration and identification of seven genera of intestinal bacteria, all potentially pathogenic. Bacteriological analyses were performed using fecal swab techniques and aquaria water of twenty-seven individually purchased specimens of Pseudemys scripta elegans. Representatives of Aeromonas, Proteus, Salmonella, Citrobacter, Enterobacter, Klebsiella and Serratia were isolated. This study indicated that Salmonella-free certification alone, is inadequate in rendering these reptiles as bacteriologically safe pets (McCoy and Seidler, 1973).

No bacterial studies have been reported on the intestinal flora of Chelydra serpentina serpentina. This is understandable since snapping turtles have been behaviorally characterized as violent, highly aggressive animals with vicious tempers. They strike with amazing speed and their jaws are capable of tearing flesh severely (Ernst and Barbour, 1972). Most of the studies of this organism have been directed toward evolutionary and behavioral characteristics.

Studies of animal-bacterial relationships have been largely determined by medical or economic needs and are therefore mostly restricted to human bacterial pathogens, insect-bacterial relationships and domestic animal bacterial retention.

The earliest work reported on bacterial retention in the alimentary tract of an insect was by Forbes in 1882 who reported the presence of bacteria in the caecae of certain Heteroptera. The first attempt to deliberately infect an organism by orally introducing a bacterium was described by Faichnie in 1909. He permitted fly larvae, Musca domestica, to feed on feces containing Bacillus typhosus and isolated this bacterium from the newly emerged adults of the colony. No attempt was made to recover the bacterium from the alimentary tract of the contaminated larvae, nor was any study conducted on the retention time of the bacterium within the larvae's digestive tract. The author reported that Bacillus typhosus survived through the metamorphosis of the flies

(Faichnie, 1909, cited by Proctor, 1964).

Bacot (1911) conducted a similar investigation using the fly, Musca domestica and the bacterium, Bacillus pyocyaneus and found the bacterial number to be maximal in the adult flies immediately after emergence.

Bacot (1914, cited by Proctor, 1964) studied the survival of bacteria in the alimentary tract of insects by infecting flea larvae with Bacillus pyocyaneus, Bacillus enteritidis, Staphylococcus aureus and Staphylococcus albus bacteria. He reported that the bacteria survived through the metamorphosis from larvae to adult.

J. T. Duncan in 1926 proposed that a bactericidal substance is formed in the alimentary canal of flies especially in the early stages of development resulting in the destruction of certain species of bacteria. The active principle is formed in the stomach, but whether it is a secretion of the gastric cells or is a result of the processes of digestion is unclear. Klinge (1930) announced that living gastric mucosa seemed to possess a special bactericidal function which was suspended only by severe functional disturbances.

Bacterial retention by an insect is also influenced by the environmental conditions of the alimentary tract, such as starvation, excessive heat, pH variability of the gut contents and other stresses (Steinhaus, 1963).

The influence of stress factors was later extended to the bacterial flora in the alimentary tract of chickens.

Shirasaka and Noriyoshi (1971) starved adult chickens for 74 hours and noted that the total number of intestinal bacteria decreased markedly. When saline suspensions of Streptococcus, Enterobacteriaceae, and Lactobacillus were introduced orally into starved chickens, retention time seemed related to the presence of nutrients in the digestive tract.

In a study by Rantala and Nurmi (1973), forty-two day old chicks were inoculated orally with Salmonella infantis, following pretreatment with the cultured flora of the alimentary tract of an adult chicken or fluids from the alimentary tract. The normal flora of the adult chicken prevented the establishment of this bacterial species in the caeca of the fowl.

Knuckles (1972) investigated the survival of enteric pathogens in the pupae of Phormia regina. Blowfly larvae were inoculated with Salmonella schottmuelleri or Salmonella typhimurium, and the pupae examined bacteriologically and serologically to determine the infectivity rate and the persistence of the pathogens. Both bacterial species persisted in the pupae for 18 to 29 days.

Current interest in bacterial retention is directed toward the mechanisms by which coproantibodies protect an organism against enteric bacterial infection. Fubara and Freter (1973) stated, "The mucosal cell may furnish some accessory factor which in conjunction with the antibody, results in an antibacterial mechanism on the mucosal surface."

This is an attempt to explain the possible mechanism of Duncan's bactericidal principle first proposed in 1926.

Serratia marcescens has been widely used as a marker organism to study bacterial retention in insects. However, its use in other animals for the same purpose, is extremely limited (Traub and Raymond, 1971).

Serratia marcescens is a small, peritrichous, gram negative rod, producing variable amounts of red pigment, prodigiosin, and may appear as non-pigmented under appropriate cultural methods. It is commonly found in water, soil and milk where it produces a blood-like discoloration (Hawker and Linton, 1971). It is a facultative anaerobe, grows in a wide range of pH, and is not nutritionally fastidious (Breed et al., 1957).

Serratia marcescens was described in 1823 by Bizio (cited by Davis, 1970) and in recent decades it has been associated with serious infections and even death in man. It has been identified as the infectious agent in lung wounds, in burns, and as one of the primary agents of urinary tract infections (Davis et al., 1970). Serratia was also cultured from the hands of hospital personnel (Maki et al., 1973).

Some types of Serratia marcescens are more prevalent than others and in humans it seems to have an affinity for elderly, seriously ill males (Traub and Raymond, 1971), sometimes causing septicemia (Davis et al., 1970).

The pathogenicity of Serratia marcescens may be

increased by its rapid rate of mutability. The antibiotic cephalosporin is effective against Serratia but resistant forms to cephalosporin by its ability to produce cephalosporinase have been reported (Mildvan et al., 1972). Heavy usage of antibiotics probably promotes the selection of Serratia and the predominance of highly resistant strains (Maki et al., 1973).

Pye and Yendol injected Serratia marcescens into the larvae of the greater wax moth and found it to be pathogenic for this insect. Death in the moths resulted from a breakdown in enzyme activity and specifically from a decrease in the ability to produce polyphenoloxidase (Pye and Yendol, 1972).

Most investigators who noted instances in which Serratia marcescens exhibited pathogenicity referred to laboratory reared insects. This suggests that the ability of this species of bacteria to cause disease may be related to factors of stress that a laboratory environment imposes and which do not prevail in nature. Steinhaus (1959) confirmed these observations and determined that Serratia marcescens was more pathogenic when injected into the hemocoel than when ingested by insects. In all cases, death of the infected insects was preceded by septicemia. At death there was considerable tissue destruction and dead insects rapidly decomposed.

Wedberg, Brandt and Helmbolt (1949) investigated the

passage of microorganisms through the digestive tract of the roach, Blaberus cranifer. Extensive multiplication of Serratia marcescens occurred in some of the roaches and were excreted within 48 hours and intermittently throughout the life of the orally-infected roach--some living up to 145 days.

A difficulty frequently encountered in using Serratia marcescens as a bacterial marker to study bacterial retention by a given organism is the relative mutability of its characteristic red pigment, prodigiosin. Production of prodigiosin by Serratia marcescens has been found to be influenced by a number of environmental conditions such as the presence of magnesium, a phosphate, a sulfate, an organic source of nitrogen, pH (Dewey and Poe, 1943), iron (Porter, 1947), ultra violet radiation (Labrum and Bunting, 1953), and temperature and oxygen requirements (Smith and Johnson, 1954).

Mutations affecting pigment production are easy to observe, but the actual biochemical changes are unknown in most cases. The mutants are almost completely stable and are distinguished by the complete loss of color or production of intermediate tints. White, rose red, and pink are three mutants of Serratia marcescens which have been defined (Carpenter, 1961). Other studies of Serratia marcescens have been directed toward pigment function (Burdon, 1958) and the relationship of metabolites to pigment production (Saban and

Lynch, 1972).

Considerable interest is directed to epidemiological factors as animals of various species are increasingly incriminated as vectors of pathogenic microbial organisms. The relationship of the bacterium, Serratia marcescens and turtles is a most interesting area for investigation. The purpose of this investigation was to determine the persistence of the bacterium Serratia marcescens in the intestinal tracts of turtles.

METHODS AND MATERIALS

The turtles used in this study were obtained from three sources. The snapping turtles, Chelydra serpentina serpentina, (two year old siblings) were obtained from the Drake University biology department. The red-eared turtles, Pseudemys scripta elegans (hatchlings), were purchased from a pet store in Des Moines, Iowa; and the western painted turtles, Chrysemys picta belli (five years old), were obtained from a commercial biological supply company in Oshkosh, Wisconsin.

Depending on availability, nine to twelve turtles of each genera were used in the study. The snapping turtles weighed approximately 46.5 gms with carapace dimensions averaging 6.5 cm wide and 7.5 cm long. The red-eared turtles weighed approximately 5.8 gms and had carapace dimensions of

2.6 cm wide and 2.6 cm long. The western painted turtles were the largest and oldest specimens used weighing an average of 343.5 gms and having carapace dimensions of 9.0 cm wide and 13.0 cm long.

The turtles were isolated and maintained at room temperature in separate tanks containing tap water. The tanks were cleaned once a day using hot tap water and "All" detergent. Each tank was provided with a half-brick and an overhanging gooseneck incandescent lamp with a 40 watt bulb for basking. All animals were fed water-softened Ken-L Ration dog meal pellets.

The bacterium, Serratia marcescens, was obtained from a stock culture at Drake University, subcultured on tryptic soy agar (Difco Laboratories) every eight days and maintained at room temperature. This bacterial species was chosen as the marker organism for this study because it is not a member of the normal bacterial flora of the intestinal tract of turtles and it is easily identified by its characteristic red pigment, prodigiosin.

Preliminary test cultures using physiological saline (0.85% NaCl), tap water, Ken-L Ration dog meal pellets, and fecal material from each genera of turtles were plated in triplicate on tryptic soy agar to establish the absence of Serratia marcescens in any of the experimental substances and to note the appearance of these materials on each type of agar. Further tests were conducted mixing Serratia marcescens with

each of the above substances and plating them in triplicate on both tryptic soy agar and an asparagine enriched agar to determine the visible effect of the experimental materials on the marker bacterium, Serratia marcescens.

All food inoculations were made from 72 hour tryptic soy broth (Difco Laboratories) cultures of Serratia marcescens which had been inoculated from the tryptic soy agar stock slants and incubated at room temperature. A one cc stylex tuberculin syringe fitted with a number 20 gauge, 1 inch stainless steel needle (Pharmaseal Laboratories, Glendale, Calif.) was used to inject 0.1 ml of the 72 hour Serratia marcescens broth culture into the center of the Ken-L Ration dog meal pellet softened with 0.9 ml physiological saline (0.85% NaCl).

One food pellet inoculated with the appropriate concentration of Serratia marcescens (Table 1) was dropped into each tank. The food was consumed in one to five seconds by the snapping turtles and the red-eared turtles. The western painted turtles had to be force fed by gently probing their jaws with the needle of the inoculation syringe until they opened their mouth. The Serratia marcescens broth culture of the same concentration as that injected into the food pellets, was then discharged directly into the mouth cavity from the syringe and no food pellets were used in these inoculations. All tanks were cleaned immediately after this feeding. The turtles were rinsed by holding them under the running tap

Table 1. Number of turtles fed dog meal pellets pre-injected with concentrated, 10^{-2}, and 10^{-4}, 72 hour Serratia marcescens.

	Trial 1				Trial 2		Trial 3		Trial 4	
Genus	Conc.	10^{-2}	10^{-4}	Control	Conc.	Control	Conc.	Control	Conc.	Control
Pseudemys	3	3	3	3	6	4	6	4	-	-
Chelydra	3	3	3	3	6	6	6	6	6	6
Chrysemys	-	-	-	-	-	-	-	-	5	4

water before replacing them in the cleaned tanks. A water sample was collected from the tanks using a sterile one ml pipet (Corning Glass Works). This sample (0.1 ml) was plated on tryptic soy agar to ascertain that no Serratia marcescens was present to contaminate the tanks.

One fecal sample was collected, if available, each day from each turtle using a sterilized L-shaped 8.0 x 24 mm glass rod. When available, the solid fecal sample was suspended in a sterile pyrex test tube (16 x 150 mm) containing 0.9 ml physiological saline (0.85% NaCl) solution. The standard pour plate technique using tryptic soy agar and an asparagine enriched agar was used for isolating organisms from the samples. The asparagine enriched agar was to provide maximum pigmentation of Serratia marcescens as reported by Dewey and Poe (1943). Duplicate plates were prepared with tryptic soy agar and the asparagine enriched agar, using 0.1 ml of concentrated and 10^{-1} fecal and tank water samples. The plates were incubated at room temperature and read for growth after 48 hours. Samples were collected over a five to nine day period for each of four trials.

RESULTS

A 48 hour culture of physiological saline (0.85% NaCl), tap water, Ken-L Ration dog meal pellets and fecal material from each genera of turtles on tryptic soy agar plates

incubated at room temperature indicated that Serratia marcescens was not present in any of these substances at the beginning of the investigation. When Serratia marcescens was added to each of the above and cultured on tryptic soy agar and asparagine enriched agar plates for 48 hours at room temperature, the bacteria was recovered from all cultures and growth was abundant. The Serratia marcescens recovered from the fecal material appeared as a light pink on tryptic soy agar and a slightly deeper flesh-pink on the asparagine enriched agar. However, when diluted 10^{-1} and subcultured on either agar, the bacteria displayed the normal red pigment, prodigiosin.

No Serratia marcescens was recovered in the first trial involving a five day observation period, when 72 hour concentrated, 10^{-2} and 10^{-4} serially diluted bacterial cultures were introduced through inoculated food pellets to the digestive tracts of three red-eared turtles and three snapping turtles for each dilution. In the second and third trials, Serratia marcescens was recovered from one of six inoculated red-eared turtles in each trial for a maximum of 5 days. In trial 2, four of the five positive samples from the red-eared turtles were recovered by day 3. In trial 3, seven of the eight positive samples for the red-eared turtles were recovered by day 2. Serratia marcescens was not recovered beyond day 3 in trial 2, beyond day 2 in trial 3, nor from any of the control fecal or water samples (Table 2).

Table 2. The recovery of *Serratia marcescens* from *Pseudemys scripta elegans* in fecal (F) and tank water (W) samples collected after the introduction of 72 hour *S. marcescens* to the digestive tracts of the turtles, expressed as samples collected/*S. marcescens* recovered.

Trial	No. of turtles	Inoculation strength	Day 1 F	Day 1 W	Day 2 F	Day 2 W	Day 3 F	Day 3 W	Day 4 F	Day 4 W	Day 5 F	Day 5 W
1	3	Conc.	1/0	0/0	3/0	0/0	1/0	0/0	0/0	0/0	1/0	0/0
	3	10^{-2}	0/0	0/0	3/0	0/0	1/0	0/0	0/0	0/0	1/0	0/0
	3	10^{-4}	0/0	0/0	0/0	0/0	0/0	0/0	2/0	0/0	0/0	0/0
	3	Control	1/0	0/0	2/0	0/0	1/0	0/0	2/0	0/0	1/0	0/0
2	6	Conc.	2/1	0/0	2/1	0/0	3/0	6/2	6/0	6/0	5/1	6/0
	4	Control	4/0	0/0	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3	6	Conc.	5/4	6/2	1/1	6/0	2/0	6/0	5/0	6/0	5/1	6/0
	4	Control	2/0	4/0	2/0	4/0	0/0	0/0	0/0	0/0	0/0	0/0

In the second, third and fourth trials using 12 snapping turtles for each trial, Serratia marcescens was recovered from 2/6 experimental turtles in trial 2 until day 5. In trial 3, the maximum retention ratio was 1/6 to day 3. In trial 4, Serratia marcescens was recovered from 1/6 experimental turtles until day 4. Nine of the 11 positive samples in trial 2 were recovered by day 4; four of the 6 positive samples in trial 3 were recovered by day 1; and 7 of the 8 positive samples in trial 4 were recovered by day 3. Serratia marcescens was not recovered from any of the control fecal or water samples (Table 3).

The western painted turtles were used for one trial. Of the five experimental turtles, Serratia marcescens was recovered from one individual on day 2. Red pigmented bacteria were not recovered from the fecal or water samples of the four control turtles (Table 4).

Considering all trials the average percent recovery of Serratia marcescens from the alimentary tract of each experimental turtle was 32.9% for the red-eared turtles, 42.9% for the snapping turtles and 20.0% for the western painted turtles. The retention time ranged from 0 to 5 days for both the red-eared turtles and the snapping turtles, and 0 to 2 days for the western painted turtles. The mean retention time in days for each of the genera investigated was 0.6 for the red-eared turtles; 1.1 for the snapping turtles, and 0.4 for the western painted turtles (Table 5).

Table 3. The recovery of *Serratia marcescens* from *Chelydra serpentina serpentina* in fecal (F) and tank water (W) samples collected after the introduction of 72 hour *S. marcescens* to the digestive tracts of the turtles, expressed as samples collected/*S. marcescens* recovered.

Trial	No. of turtles	Inoculation strength	Day 1 F	Day 1 W	Day 2 F	Day 2 W	Day 3 F	Day 3 W	Day 4 F	Day 4 W	Day 5 F	Day 5 W
1	3	Conc.	2/0	0/0	3/0	0/0	1/0	0/0	0/0	0/0	2/0	0/0
	3	10^{-2}	0/0	0/0	3/0	0/0	2/0	0/0	0/0	0/0	1/0	0/0
	3	10^{-4}	3/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
	3	Control	1/0	0/0	2/0	0/0	1/0	0/0	0/0	0/0	0/0	0/0
2	6	Conc.	1/0	0/0	3/2	0/0	3/1	6/4	4/1	6/1	5/0	6/2
	4	Control	1/0	0/0	1/0	0/0	5/0	6/0	1/0	6/0	0/0	6/0
3	6	Conc.	3/2	6/2	3/1	6/1	3/0	6/0	6/0	6/0	0/0	6/0
	6	Control	4/0	6/0	4/0	6/0	0/0	0/0	0/0	0/0	0/0	0/0
4	6	Conc.	3/1	6/3	4/1	6/1	1/0	6/1	2/0	6/1	4/0	6/0
	6	Control	4/0	6/0	2/0	6/0	0/0	0/0	0/0	0/0	0/0	0/0

Table 4. The recovery of *Serratia marcescens* from *Chrysemys picta belli* in fecal (F) and tank water (W) samples collected after the introduction of 72 hour *S. marcescens* to the digestive tracts of the turtles expressed as samples collected/*S. marcescens* recovered.

Trial	No. of turtles	Inoculation strength	Day 1 F	Day 1 W	Day 2 F	Day 2 W	Day 3 F	Day 3 W	Day 4 F	Day 4 W	Day 5 F	Day 5 W
1	5	Conc.	4/0	5/0	3/0	5/1	4/0	5/0	4/0	5/0	4/0	5/0
	4	Control	4/0	4/0	4/0	4/0	0/0	0/0	0/0	0/0	0/0	0/0

Table 5. Percent recovery and mean retention time of *Serratia marcescens* in the digestive tracts of three genera of turtles given food inoculated with 72 hour *S. marcescens*.

	Pseudemys	Chelydra	Chrysemys
Experimental turtles per trial	7	7	5
No. of turtles excreting *S. marcescens* per trial	2.3	3	1
Percent recovery per turtle	32.9	42.9	20.0
Retention time range in days	0 - 5	0 - 5	0 - 2
Mean retention time per turtle in days	0.6	1.1	0.4

Throughout this investigation, the quantity of Serratia marcescens recovered from any of the experimental turtles, never exceeded two colonies per plate. All of the recovered Serratia marcescens were isolated on tryptic soy agar, except two colonies which were isolated on asparagine enriched agar. All sampling from day 6 to day 9 was negative for Serratia marcescens.

The correlation between the average number of turtles of the three genera investigated, excreting Serratia marcescens after the introduction of the bacteria to the turtle's digestive tracts and the consecutive fecal and/or water samples recovered is illustrated in Figure 1.

None of the turtles inoculated with Serratia marcescens showed any signs of illness after the introduction of the bacterial organisms. All turtles, both the experimental and the controls continued to eat normally and were typically active throughout the investigation.

DISCUSSION

Under the conditions established for this investigation it is suggested that Serratia marcescens cannot withstand the environmental conditions of the gastro-intestinal tract of the three genera of turtles studied (Figure 1). The presence of bactericidal substances as reported by Duncan (1926), the influence of coproantibodies in inhibiting

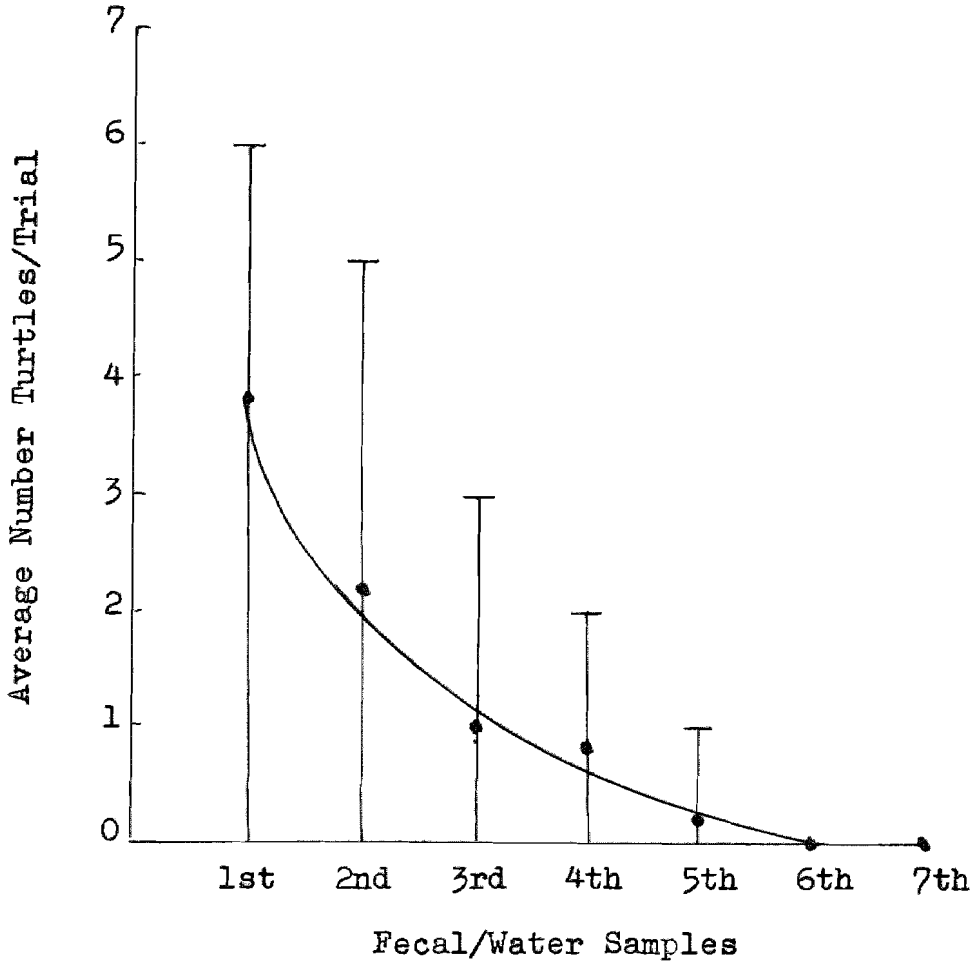

Figure 1. Correlation between the average number of three genera of turtles per trial excreting Serratia marcescens after tne introduction of the bacterium to the turtles' digestive tracts through inoculated food, and the consecutive fecal and/or water samples recovered.

bacterial adsorption to the intestinal mucosa (Fubara and Freter, 1973), the nature of the food ingested, the hydrogen ion concentration which varies from 2.2 - 9 in the digestive tracts of turtles (Fox and Musacchia, 1959), the moisture content of the intestinal tract, the digestive fluids and secretions, the inhibitory lysozyme which may act synergically with trypsin, the frequency of defecation, and the nature of the intestinal wall, may to some degree account for the inability of Serratia marcescens to survive the gastrointestinal tracts of Pseudemys, Chelydra, and Chrysemys for long periods of time.

Intestinal motility may be an important limiting factor in the retention of Serratia marcescens in the turtle's digestive tract. In this study, several turtles were observed consuming their own feces immediately after depositing it. Apparently food material is passed through the alimentary tract before it is completely digested possibly retaining some nutritional value. This may explain the appearance of one or two colonies of Serratia marcescens in the few positive fecal cultures recorded in Tables 2, 3 and 4. The bacteria may have escaped the bactericidal activity of the intestinal environment by being embedded in a food particle which passed through undigested. This assumption is supported by the observation that Serratia marcescens was recovered from some tank water samples and not from subsequent fecal samples from the same turtles.

When bacteria are exposed to a physical or chemical agent inimical to their survival, death usually does not occur instantaneously to the entire population. Rather, the death rate for a particular organism follows a definite predictable pattern. Under uniform conditions the number of organisms will be reduced by the same percentage during each equal period of time (Pelczar and Reid, 1965). By using the bacterial retention time as an indication of bacterial survival in the intestinal tracts of the experimental turtles, this predictable pattern of death of the bacteria seems to be supported by the data shown in Figure 1.

The zero retention time of trail 1 (Tables 2, 3 and 4) is probably a result of the initial difficulty experienced in attempting to successfully introduce the marker bacterium, Serratia marcescens, to the turtles. Some of the turtles had to be force fed by directly depositing the bacterial suspension in the mouth cavity. By using this technique, the Serratia, unprotected by surrounding food, may have been exposed to enzymatic destruction in the mouth as well as the gastro-intestinal chemical digestion. This may also account for the limited persistence of Serratia marcescens in the western painted turtles in trial 4 (Table 4).

Being much smaller in size, the red-eared turtles tended to macerate the inoculated food balls to a much greater extent than the larger snapping turtles. This may explain the increased bactericidal activity of the red-eared turtles

as compared to the snapping turtles (Tables 2, 3 and 5).

It could be assumed that the bactericidal capacity of the digestive tracts of turtles would be high due to their scavenger feeding habits. Although turtles are known to be omnivorous with sidely diversified nutritional requirements (Carr, 1952; Clark and Gibbons, 1969; Feuer, 1971), Pell (1940) reported a preference for carrion over live fish in his study of snapping turtles captured along the eastern coast of the U.S. An efficient bactericidal capacity would likely be an essential survival factor in such animals.

Adverse temperatures and crowding have been reported as having a debilitating effect on organisms and possibly rendering them more susceptible to infection. Neither of these factors appear valid under the conditions of this investigation for the turtles used. Adequate provision for body temperature modification and regulation was supplied for these poikilothermic animals by the incandescent lamp left on for the duration of the entire study. The basking habit of turtles may also directly affect the persistence of bacterial invaders since the resultant elevation of body temperature would raise the rate of phagocytic activity (Board and Fuller, 1974). Each time the tanks were cleaned (once each day) they were refilled with cold tap water. This provided an additional opportunity for thermoregulation. Since each of the turtles were housed in a separate tank, population crowding is also overruled as a stress factor.

However, as the normal migration range of these turtles is much greater than the confines of the tanks used in the study, some stress may have been present. Nevertheless, the ability of these animals to resist the bacterial invasion of Serratia marcescens indicates, that if present, this crowding factor was insignificant in determining individual susceptibility to the potential pathogen.

Serratia marcescens is apparently non-pathogenic in turtles. As reported earlier, no signs or symptoms of illness or homeostatic disturbance was observed at any time during this investigation. The fecal material of the experimental turtles showed no macroscopic variation from the feces of the control turtles. When plated on tryptic soy agar or asparagine enriched agar, similar quantities and varieties of normal bacterial flora were isolated from all the turtles. In view of the fact that Serratia marcescens has been reported as an insect pathogen when present in sufficient numbers (Bucher, 1960; Proctor, 1964), it can be implied from this investigation that the pathogenicity of S. marcescens is host-dependent and is quite variable from one host to another.

This study indicates that the turtle is probably not a vector for potential pathogens when held in a tank for five days. However, this response does not preclude the possibility that turtles may be vectors for other human pathogens. Further studies are needed to determine the retention

time of known human bacterial pathogens in turtles before these Chelonians may be considered as pathogen-free pets.

SUMMARY

A study was made to determine the persistence of the bacterial species, Serratia marcescens, in the intestinal tracts of three species of turtles. The bacterium was introduced by injecting it into a food pellet and feeding this inoculated food to the experimental turtles or by direct inoculation of the bacterial culture into the mouth of the turtles without the use of food pellets. Control turtles were maintained under similar conditions without bacterial inoculation. Retention time of the Serratia marcescens by the turtles was measured by collecting fecal and tank water samples once a day for five to nine days and plating these samples on tryptic soy agar and an asparagine enriched agar. All plates were incubated at room temperature and read after 48 hours. The experiments showed that Serratia marcescens produced no visible changes or symptoms in the turtles and the bacterial species was unable to be demonstrated in fecal materials from the turtles after five days.

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